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Simulation of the Outer Membrane Protein X in a Lipid Bilayer and in a Micelle

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The outer membrane protein X (OmpX) from *Escherichia coli* was simulated embedded in a phospholipid bilayer and as a protein-micelle aggregate. The resulting simulation trajectories were analyzed in terms of structural and dynamical properties of the membrane protein (MP). In agreement with experimental observations it was found that the β-barrel region, embedded in the lipophilic phase, is very stable, whereas the extracellular, protruding β-sheet, that plays an important role in cell adhesion and invasion of gram negative bacteria, shows large structural fluctuations. Additionally, water permeation into the core of the β-barrel protein was investigated: a very stable salt-bridge and hydrogen-bond network exists in that barrel and a water flux is therefore unlikely. No great difference in protein stability and dynamics between the bilayer and the micellar systems were observed.

1 Introduction

Membrane proteins (MPs) fulfill a wide spectrum of biological functions and it is estimated that MPs constitute 30 % of all proteins in living organisms. Furthermore, numerous diseases are directly related to MPs and more than 70 % of all currently available drugs are estimated to act via MPs. However, compared to water soluble proteins, structural information for MPs is sparse. Molecular dynamics (MD) simulation offers the possibility to describe the dynamic behavior of MPs. Additionaly, it is possible to compare the dynamics of a MP in the experimental environment, i.e. in protein-detergent co-crystals in X-ray diffraction experiments and in protein-micele aggregates in NMR solution-experiments, to its dynamics when embedded in a lipid bilayer.

OmpX belongs to the family of outer membrane proteins (Omp) of gram-negative bacteria and represents one of its smaller members. Its structure was studied by X-ray crystallography[1] and in a mixed protein-lipid micelle by TROSY NMR spectroscopy[2]. OmpX is characterized by eight antiparallel β-strands connected by three periplasmic turns and four extracellular loops (Figure 1). Four of the eight β-sheets protrude into the extracellular space, thus this β-sheet, also denoted as "waving flag", was suggested to act as a hydrogen bonding partner to other proteins in the extracellular space with complementary β strands at their surfaces[1]. This structural property of OmpX confirms conclusions drawn from microbiological studies that OmpX plays a key role in cell adhesion and mammalian cell invasion.

Here, OmpX was studied by MD when embedded in a lipid bilayer and as a protein-micelle aggregate. Experimental observations concerning the flexibility of the protruding β-sheet, the degree of stability of the hydrogen-bond network in the interior of the protein and the (in-)ability of OmpX to act as water pore can be ideally complemented with simulation studies at the atomic level.

2 Simulation Setup

The OmpX protein was simulated in a lipid bilayer, consisting of dimyristoyl-phosphatidylcholine (DMPC) molecules, and in a dihexanoyl-phosphatidylcholine (DHPC) micelle. The NMR structure[2] was employed as a starting structure. Two simulations, where OmpX was inserted in a lipid bilayer (simulations OmpX-DMPC-1 and OmpX-DMPC-2) and one simulation of the micellar protein-lipid aggregate (simulation OmpX-DHPC) were performed. The protein in the simulation OmpX-DMPC-1 initially contains no water in the internal cavities of OmpX, while in OmpX-DMPC-2, as well as in OmpX-DHPC, these cavities were initially filled with water (Table 1). All simulations were performed using the GROMOS simulation software[3] and the GROMOS biomolecular force field (version 45A3_CH95)[3,4].

system/simulation name	OmpX-DMPC-1	OmpX-DMPC-2	OmpX-DHPC
lipid and assembly type	DMPC, bilayer	DMPC, bilayer	DHPC, micelle
number of lipids	104	104	82
number of water molecules	6518	6559	12682
number of counter-ions	2 Na^+	2 Na^+	2 Na^+
total number of atoms	25876	25999	42044
type of box	rectangular	rectangular	truncated octahedron
box size [nm^3]	$5.8\times6.3\times9.2$	$5.8\times6.3\times9.5$	9.9–9.9–9.9
equilibration time [ns]	0.44	0.44	0.3
simulation (production) time [ns]	15	25	25

Table 1. Details of the simulation setups.

3 General Structural Analysis

In all three simulations, the root-mean-square-deviation (RMSD) of the protein from the initial NMR structure is rather high. This high RSMD is mainly affected by the extracellular loops, which are completely exposed to the solvent and show very large root-mean-square-fluctuations (RMSF). In contrast, the β-barrel and the periplasmic turns appear to be rather stable.

61 to 64 % of OmpX residues are on average observed to adopt a β-strand-like conformation in the simulations, while in the X-ray structure the β-strand content is higher (78%). The difference mainly originates from the the larger RSMF of the protruding β-sheet of OmpX in the simulations; while in the crystal, this β-sheet is involved in interactions with a neighboring OmpX molecule. In contrast, the barrel behaves as a rather rigid entity in the simulations as well as in the X-ray and NMR experiments.

4 Sturdy β-Barrel and Flexible "Waving Flag"

The overall agreement between NOE distances inferred from simulation and experiment is satisfactory. Simulations fulfill 78-85 % of the experimentally derived NOE distances and 52-60 % of all violations do not exceed 0.1 nm. In the extracellularly protruding β-sheet regions, only a few interstrand NOEs could be unambiguously assigned, and fast amide proton exchange was observed in the NMR solution experiments[2]. The "waving flag" seems therefore to be experiencing a large plasticity and a local fraying. The relatively large isotropic atomic B-factors derived from the X-ray diffraction data[1] of the protruding β-sheet are consistent with the NMR derived observations. However, the few NOE distances available for this region are not very well reproduced in the simulations, indicating that the protruding β-sheet region is somewhat too mobile in the simulations. The region of this four β-strands involved in the protruding β-sheet might show frequent transitions between hydrogen-bonded folded structure and solvent-exposed less secondary defined structure.

5 Water Exchange in the β-Barrel

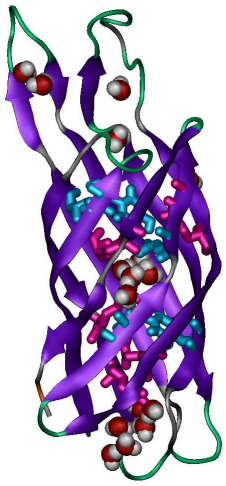

Figure 1. OmpX protein after a simulation of 25 ns in a micelle. The different colors indicate the secondary structure assignment: β-sheets are in violet, turns are in green, random coils are in white. Residues making hydrogen bonds to another residue for more than 45 % and more than 85 % of the simulation time are drawn respectively in blue and in pink. Water molecules are represented as red van der Waals spheres with grey hydrogens.

Most side-chains of polar and charged residues of OmpX point to the interior of the β-barrel and form a network of rather stable hydrogen bonds and salt-bridges (Figure 1). Consequently, no pathway exists between the extracellular and the periplasmic end of the barrel, making it unlikely to observe a continuous water flux through the barrel. Nevertheless, in the simulations of OmpX-DMPC-2 and OmpX-DHPC, some exchange between the internal water cavities and bulk water is observed.

6 Bilayer Versus Micelle Environment

From the data presented here, no significant differences in protein stability or dynamics can be detected between the simulations in a DMPC bilayer and a DHPC micelle. However, when comparing the few interstrand NOEs of the protruding β -sheet, the protein-micelle simulation appears to fulfill the long-range NOEs better than the simulations of the protein-bilayer system. The extracellular loops seem as well to have even more structural freedom in a bilayer system than in a micellar system.

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